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UTILITY PATENT APPLICATION TRANSMITTAL *(Only for new nonprovisional applications under 37 C.F.R. 1.53(b))*	Attorney Docket No.	2825.1013002
	First Named Inventor or Application Identifier	Hiten D. Madhani
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Title of Invention	TARGETS OF THE MAP KINASE PATHWAY IN THE DEVELOPMENTAL SWITCH IN YEAST	

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APPLICATION ELEMENTS
See MPEP chapter 600 concerning utility patent application contents.

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1. [ ] Fee Transmittal Form
(Submit an original, and a duplicate for fee processing)
2. [X] Specification **[Total Pages [16]]**
(preferred arrangement set forth below)
- Descriptive title of the invention
- Cross References to Related Applications
- Statement Regarding Fed sponsored R & D
- Reference to microfiche Appendix
- Background of the Invention
- Summary of the Invention
- Brief Description of the Drawings
- Detailed Description
- Claim(s)
- Abstract of the Disclosure
3. [XX] Drawing(s) (35 U.S.C. 113) **[Total Sheets [11]]**
[ ] Formal [XX] Informal
4. [ ] Oath or Declaration/POA **[Total Pages [ ]]**
a. [ ] Newly executed (original or copy)
b. [ ] Copy from a prior application (37 C.F.R. 1.63(d))
(for continuation/divisional with Box 17 completed)
[NOTE Box 5 below]
i. [ ] DELETION OF INVENTOR(S)
Signed statement attached deleting inventor(s) named in the prior application, see 37 C.F.R. 1.63(d)(2) and 1.33(b).
5. [ ] Incorporation By Reference *(useable if Box 4b is checked)*
The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied under Box 4b, is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein.
6. [ ] Microfiche Computer Program *(Appendix)*
7. [ ] Nucleotide and/or Amino Acid Sequence Submission (if applicable, all necessary)
a. [ ] Computer Readable Copy
b. [ ] Paper Copy (identical to computer copy)
[ ] **Pages**
c. [ ] Statement verifying identity of above copies

ACCOMPANYING APPLICATION PARTS

8. [ ] Assignment Papers (cover sheet & documents)
9. [ ] 37 C.F.R. 3.73(b) Statement *(when there is an assignee)* [ ] Power of Attorney
10. [ ] English Translation Document *(if applicable)*
11. [ ] Information Disclosure Statement (IDS)/PTO-1449 [ ] Copies of IDS Citations
12. [ ] Preliminary Amendment
13. [X] Return Receipt Postcard (MPEP 503) (2)
(Should be specifically itemized)
14. [ ] Small Entity Statement(s) [ ] Statement filed in prior application, status still proper and desired
15. [ ] Certified Copy of Priority Document(s)
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16. [ ] Other: ______

17. **If a CONTINUING APPLICATION,** check appropriate box and supply the requisite information:
[ ] Continuation [ ] Divisional [ ] Continuation-in-part (CIP) of prior application No.:
Prior application information: Examiner: Group Art Unit:

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Attorney's Docket No.: 2825.1013002

TARGETS OF THE MAP KINASE PATHWAY IN THE DEVELOPMENTAL SWITCH IN YEAST

RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application 60/108,399, filed November 13, 1998, and U.S. Provisional Application 60/114,849, filed January 6, 1999. The entire teachings of these applications are incorporated herein by reference.

GOVERNMENT SUPPORT

Work described herein was supported, in whole or in part, by National Institutes of Health Grant Number GM 40266. The United States Government has certain rights in the invention.

BACKGROUND OF THE INVENTION

Two related developmental events, haploid invasive growth and diploid pseudohyphal development, are controlled by the Kss1 MAP kinase pathway in yeast. Haploid invasive growth occurs on rich medium, whereas filamentation in the diploid cell type requires nitrogen starvation. The diploid pathway results in dramatic cell elongation, which is not seen in haploids. These pathways serve as models for similar transitions in pathogenic fungi.

SUMMARY OF THE INVENTION

Wild yeast are often found in association with plants, particularly rotting fruit. Not surprisingly, many bacterial and plant fungal pathogens secrete pectin-degrading enzymes, including polygalacturonases. These are thought to be key virulence factors. In the bacterial pathogen *Erwinia chrysanthemi* there exists an elaborate interaction between the host and the pathogen in which the breakdown product of pectin, galacturonic acid, signals large changes in the expression of the pectinolytic machinery. To examine whether a similar interaction with the host might be occurring in yeast, global profiling experiments of gene expression in the presence of polygalacturonic acid or galacturonic acid were carried out.

Described herein is assessment of targets of the MAP kinase (MAPK) pathway in the developmental switch between haploid invasive growth and diploid pseudohyphal development in yeast, and identification of genes that show strong regulation by a MAPK pathway-specific transcription factor, Tec1. Also described herein are results of examination of expression profiles after administration of polygalacturonic acid (the main component of pectin) or galacturonic acid (the breakdown product of pectin), as well as the results of detailed studies of PGUI, a pectinase, which was shown to be the most strongly regulated target of the MAPK pathway.

Also described are global profiling experiments of gene expression in yeast which were carried out to examine whether a host-yeast interaction occurs in which the breakdown product of pectin signals or causes changes in the expression of components of the pectinolytic machinery. As discussed herein, results of these profiling experiments showed that both polygalacturonic acid and galacturonic acid altered gene expression in yeast, and that the patterns were distinct from those that would have been expected from the effects of all other sugars that have been studied in yeast (e.g., glucose, galactose, maltose, etc.), demonstrating the specificity of the response. Galacturonic acid, the breakdown product of pectin, was shown to cause strong repression of TOT10/YEL033W, a gene which is turned on in the filamentation MAPK pathway and is required for invasion and filamentation. Thus, a regulatory circuit in

yeast, in which a signal from the host (in the form of or mediated by galacturonic acid) feeds back on the filamentation/invasion pathway, has been identified, and a specific interaction between yeast and its host (e.g., a plant host) has been demonstrated for the first time.

As a result of the work described herein, targets of the MAPK pathway in fungi (e.g., yeast) and, particularly, genes that show strong regulation by Tecl, a MAPK pathway-specific transcription factor, have been identified. These genes and their interaction with or regulation by Tecl can be targeted in a method of modulating (inhibiting or enhancing) the developmental switch between haploid invasive growth and diploid filamentation. Compounds or molecules which modulate these genes, directly or through their regulation by Tecl, can be identified by means, for example, of an assay in which one or more of the genes (e.g., a gene encoding PGUI) is expressed in an appropriate host cell and the effects of a candidate modulator (inhibitor or enhancer) on its expression are determined. Candidate modulators shown to decrease expression are inhibitors of a gene shown, as described herein, to be regulated by Tecl; candidate modulators shown to increase expression are enhancers of such a Tecl-regulated gene. In addition, the TOT10/YEL033W gene, shown, as described herein, to participate in a regulatory circuit between yeast and a host (e.g., a plant host) can be targeted to modulate (decrease or increase) yeast-host interaction. It can be targeted, for example, to inhibit yeast invasion and/or filamentation and, thus, to inhibit adverse effects of fungi, including pathogenic and nonpathogenic yeast. Inhibitors (or enhancers) of TOT10/YEL033W can be identified, for example, in an assay in which the gene is expressed in an appropriate host cell and the effects of candidate inhibitors (or enhancers) are assessed. Inhibition of TOT10YEL033W, directly or indirectly (e.g., by inhibiting a gene or the product of a gene with which TOT10/YEL033W interacts) will result in inhibition of invasion and/or filamentation. Inhibitors and enhancers of genes regulated by Tecl and inhibitors of TOT10/YEL033W are the subject of this invention.

Compounds or molecules which activate or inhibit PGUI can also be identified. For example, activators of this pectinase can be identified by expressing PGUI in an

appropriate host cell (e.g., a bacterial or yeast cell), contacting the cells with (e.g., by culturing them in the presence of) candidate activators (compounds or molecules to be assessed for their effects on PGUI activity) and determining their effect on PGUI (e.g., whether they enhance or activate PGUI expression or activity, repress or decrease PGUI expression or activity or have no effect). Compounds which enhance or activate PHUI expression or activity are activators; those which repress or decrease its expression or activity are inhibitors). Activators and inhibitors of PGUI are also the subject of this invention.

Also the subject of this invention is a method of inhibiting (totally or partially) invasion of a host, particularly a plant host by a fungus (i.e., a method of inhibiting fungal invasion of a host). In the method, a compound or molecule which inhibits the MAPK pathway or specifically inhibits TOTI0/YELO33W is applied to a host (e.g., by application to a plant surface) in such a manner that it contacts the fungus (e.g., the yeast) and inhibits one or more components of the MAPK pathway, such as TOT10/YELO33W. For example, an inhibitor can be a compound which binds and inhibits TOT10/YELO33W; galacturonic acid; or a mimic of galacturonic acid which represses TOT10/YELO33W. In a specific embodiment, the method of inhibiting fungal invasion of a host comprises contacting a fungus (e.g., a yeast) with a compound which inhibits the MAPK pathway and/or inhibits TOT10/YELO33W, in sufficient quantity that inhibition of the MAPK pathway and/or inhibition of TOT10/YELO33W occurs, thereby inhibiting fungal invasion of the host. In a further embodiment, the host is a plant and the compound is applied to a plant surface (e.g., root, leaf, stem) or seed in such a manner that it contacts the fungus and inhibits (totally or partially) the ability of the fungus to invade.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows genetic expression profiles of 18 genes regulated by the filamentation MAPK pathway.

Figure 2 lists MAPK pathway targets.

Figure 3 summarizes results of systematic knockout experiments.

Figure 4 is a photograph of results of an assay showing that filamentation MAPK pathway controls pectinolysis via PGUI.

Figure 5 shows genes selectively induced by the plant-specific carbohydrate polygalacturonic acid and its hydrolysis product.

Figure 6 shows genes selectively repressed by the plant-specific carbohydrate polygalacturonic acid and its hydrolysis product.

Figure 7 is a compilation of MAPK data, sorted as TEC1-high copy/tec1Δ.

Figure 8 shows results of profiling experiments with polygalacturonic acid (PGA) and galacturonic acid (GA), sorted by PGA/YPD.

Figure 9 shows results of profiling experiments with polygalacturonic acid (PGA) and galacturonic acid (GA), sorted by GA/YPD.

Figure 10 shows a flow chart of homologous genes induced by the filamentation and mating MAPK pathways.

Figure 11 shows a listing of genes whose expression is reduced in STE12$^-$, STE7$^-$ but show greater than double an effect with Tec1.

DETAILED DESCRIPTION OF THE INVENTION

Described herein is work carried out to identify and study the targets of the MAP kinase pathway in order to understand how signaling cascades control a developmental switch in this *Saccharomyces cerevisiae* model system. The pathway consists of four kinases Ste20 (PAK), Ste11 (MEKK), Ste7 (MEK) and Kss1 (MAPK), which display both positive and negative control over the pathway, as well as a heterodimeric transcription factor Tec1-STE12. STE7, STE11 and STE20 also participate in the yeast mating MAPK pathway. Global expression patterns in haploid cells under rich medium conditions were examined in the following mutants: wild type tec1Δ Ste12Δ, Ste7Δ, TEC1-overexpression, and STE11-4 (an activated mutant of the MEKK). Expression profiling was carried out using nucleic acid arrays (chips) such as described in

WO95/11995. One chip set was used per sample (chips with obvious defects were redone).

18 genes were identified that show strong regulation by the pathway-specific transcription factor Tec1 (i.e. 3.5-20X difference in expression comparing TEC1-overexpression to tec1Δ). Almost all of these also show a consistent dependency on STE7, STE7, and STE12. One gene that was known previously to be regulated by the pathway, FLO11 (which encodes a cell surface protein required for pseudohyphal growth) is the second-most strongly regulated target. Detailed studies were performed on one of these targets, PGU1, which encodes a secreted carbohydrate-destroying enzyme. This enzyme breaks down a key component of plant cell walls, polygalacturonic acid (which is the main component of pectin).

Remarkably, galacturonic acid, the breakdown product of pectin, causes the strong repression of a gene, TOT10/YEL033W, which is turned on in the filamentation MAPK pathway and which these results have shown is required for invasion and filamentation. Thus, work described herein has identified a new regulatory circuit in yeast in which a signal from the host feeds back on the filamentation/invasion pathway. This is the first demonstration of a specific interaction between yeast and its plant host. Figures 1-11 show the data in detail.

Work described herein provides an analysis of data from haploid strains grown in rich medium conditions, and in diploid cells under nitrogen starvation conditions; that is, the conditions that promote pseudohyphal cells. Portion os this work was carried out to assess whether pseudohyphal cells respond to MAPK signaling differently compared to haploid cells. The experiments described compare the expression of strains overexpressing the transcription factor Tec1 to those lacking it. They extend the assessment of targets of the MAP kinase pathway in a yeast developmental switch in haploid cells to examination of signaling in diploid cells. The data (Tables 1 and 2) were analyzed using a floor of 20 and a maximum-minimum filter of 80. Genes showing a greater than two-fold change in duplicate samples are listed. The results indicate that a largely different set of genes is induced by the MAPK

pathway during pseudohyphal conditions. One striking exception is the *FLO11* gene, which is the gene most strongly induced both in haploids and diploids by the pathway. The other genes fall mainly into the categories of cell-cycle regulated genes (such as histones and PCNA), nitrogen scavenging factors (e.g., Dur3, Car2). A number of other genes are regulated that do not at present fit into any pattern.

Accordingly, the invention relates to a method of inhibiting invasion of a host by a fungus, comprising contacting the fungus with a compound which inhibits expression of a gene expressed in the filamentation MAPK pathway and which enhances the filamentation MAPK pathway, in sufficient quantity that inhibition of the expression of the gene occurs, thereby inhibiting invasion of the host by the fungus. In one embodiment, the host is a plant, and the compound is applied to a plant surface (e.g., a leaf, a root, a stem, a flower) in such a manner that it contacts the fungus. An effective amount of the compound can be determined empirically by assessing expression levels of the gene to be inhibited. In a preferred embodiment, the gene is TOT10/YELO33W. In one embodiment, the fungus is a yeast, such as *Saccharomyces cerevisiae*.

Agents for use in the methods of the invention include nucleic acid molecules (e.g., antisense), polypeptides and proteins, antibodies and small organic molecules. Suitable formulations of agents for use in this invention can include, for example, powders, liquids, aerosols, gels and other formulations known to the skilled artisan. The present invention also pertains to pharmaceutical compositions comprising agents identified according to the invention for use in the treatment of fungal invasion. For instance, the agent identified according to the present invention can be formulated with a physiologically acceptable medium to prepare a pharmaceutical composition. The particular physiological medium may include, but is not limited to, water, buffered saline, polyols (e.g., glycerol, propylene glycol, liquid polyethylene glycol) and dextrose solutions. The optimum concentration of the active ingredient(s) in the chosen medium can be determined empirically, according to procedures well known to medicinal chemists, and will depend on the ultimate pharmaceutical formulation

desired. In organisms other than plants, methods of administration of pharmaceutical compositions include, but are not limited to, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, oral and intranasal. Other suitable methods of introduction can also include rechargeable or biodegradable devices and slow release polymeric devices. The pharmaceutical compositions of this invention can also be administered as part of a combinatorial therapy with other agents.

The invention also relates to a method of inhibiting invasion of a host by a fungus, comprising contacting the fungus with a compound which alters activity of a gene product encoded by a gene expressed in the filamentation MAPK pathway, in sufficient quantity that alteration of the activity of said gene product occurs, thereby inhibiting invasion of the host by the fungus. For example, if the gene is one whose expression enhances (e.g., increases or potentiates) the filamentation MAPK pathway (e.g., a positive regulator of the pathway), then the compound should inhibit the expression of that gene. As used herein, inhibition is intended to included both qualitative and quantitative reduction, including complete abolishment. Conversely, if the gene is one whose expression inhibits (e.g., decreases or interferes with) the filamentation MAPK pathway (e.g., a negative regulator of the pathway), then the compound should enhance the expression of that gene. As used herein, enhancement is intended to include any qualitative or quantitative increase. For example, the gene can be TOT10/YELO33W.

Expression vectors for use in the invention typically contain a nucleic acid sequence of a gene of interest operably linked to at least one regulatory sequence. "Operably linked" is intended to mean that the nucleotide sequence is linked to a regulatory sequence in a manner which allow expression of the nucleic acid sequence. Regulatory sequences are art-recognized and can be selected according to the host cell and type of expression (e.g., constitutive) to be obtained. Accordingly, the term "regulatory sequence" includes promoters, enhancers, and other expression control elements which are described in Goeddel, *Gene Expression Technology: Methods in Enzymology 185*, Academic Press, San Diego, CA (1990). It should be understood that

the design of the expression vector may depend on such factors as the choice of the host cell to be tranformed and/or the type of protein desired to be expressed.

Prokaryotic and eukaryotic host cells transfected by the described vectors are also provided by this invention. For instance, cells which can be transfected with the vectors of the present invention include, but are not limited to, bacterial cells such as *E. coli*, insect cells (baculovirus), or mammalian cells such as Chinese hamster ovary cells (CHO). Ligating the polynucleotide sequence into a gene construct, such as an expression vector, and transforming or transfecting into hosts, either eukaryotic (avian, insect or mammalian) or prokaryotic (bacterial cells), are standard procedures (see, for example, Broach, *et al.*, *Experimental Manipulation of Gene Expression*, ed. M. Inouye (Academic Press, 1983) p. 83; *Molecular Cloning: A Laboratory Manual*, 2nd Ed., ed. Sambrook *et al.* (Cold Spring Harbor Laboratory Press, 1989) Chapters 16 and 17).

The invention also relates to a method of identifying an agent which inhibits the filamentation MAPK pathway in a fungus, comprising the steps of providing an expression vector comprising a nucleic acid molecule of a gene which is expressed in the filamentation MAPK pathway; transforming a suitable host cell with said expression vector under conditions suitable for expression of said gene contacting said host cell with an agent to be tested; and comparing the expression of said gene in the presence of the agent with the expression of said gene in the absence of said agent, wherein if the expression of said gene is lower in the presence of the agent than in the absence of the agent, then the agent is an inhibitor of the filamentation MAPK pathway in a fungus. In one embodiment, the gene is TOT10/YELO33W.

Genes which are expressed in the filamentation MAPK pathway can be identified by standard methods in the art. In one embodiment, the gene is identified by expression profiling as having repressed expression in the presence of galacturonic acid as compared with in the absence of galacturonic acid. In another embodiment, the gene can be identified by expression profiling as being expressed in haploid fungal cells and not expressed in diploid fungal cells, or as being repressed by Tec1 expression.

The invention also relates to a method of inhibiting fungal filamentation, comprising contacting the fungus with a compound which inhibits expression of a gene expressed in the filamentation MAPK pathway, in sufficient quantity that inhibition of the expression of the gene occurs, thereby inhibiting filamentation by the fungus.

The invention further relates to a method of identifying an agent which modulates PGUI gene expression, comprising the steps of providing an expression vector comprising a nucleic acid molecule encoding PGUI; transforming a suitable host cell with said expression vector under conditions suitable for expression of PGUI; contacting said host cell with an agent to be tested; and comparing the expression of PGUI in the presence of the agent with the expression of PGUI in the absence of said agent, wherein a difference in the expression of PGUI in the presence of the agent as compared with in the absence of the agent indicates that the agent modulates PGUI expression.

The invention also includes a method of reducing the adverse effects of fungal invasion of a host, comprising administering to the host an effective amount of an agent which inhibits PGUI expression in the fungus.

The invention further includes a method of inhibiting invasion of a host by a fungus, comprising contacting the fungus with a compound which enhances expression of a gene expressed in the filamentation MAPK pathway and which inhibits the pathway, in sufficient quantity that enhancement of the expression of the gene occurs, thereby inhibiting invasion of the host by the fungus.

While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the spirit and scope of the invention as defined by the appended claims.

Table I

PSEUDOHYPHAL CONDITIONS

gene	Information
FLO11 (YIR019C)	GPI-anchored cell surface flocculin req'd for invasion
DUR3 (YHL016C)	Urea Permease
HTA2 (YBL003C)	Histone H2A
HTB2 (YBL002W)	Histone H2B
ORF YNL300W	GPI-anchored S/T rich protein
ORF YOL162W	Allantoate permease family
ORF YLL057C	Similar to E. coli taurine dioxygenase
SVS1 (YPL163C)	S/T rich protein req'd for vanadate resistance
ORF YOL163W	Allantoate permease familhy
CAR2 (YLR438W)	Ornithine aminotransferase, arginine catabolism
TSL1 (YML100W)	Trehalose-6-phosphate synthase/phosphatase subunit
PRY2 (YKR013W)	Homolog of Plant Pathogen-Induced Gene
POL30 (YBR088C)	PCNA, DNA Replication, Repair and Cell Cycle Factor
PDC6 (YGR087C)	Pyruvate decarboxylase: isobutyl alcohol formation
ORF YOR247W	S/T rich protein related to Svs1

Floor=20, max-min>80,max/min>2, TEC1HC/*tec1*Δ>2 for both chip sets

Table 2

PSEUDOHYPHAL CONDITIONS

gene	tec1KO A	TEC1HC A	tec1KO B	tec1HC B
FLO11 (YIR019C)	46	505	61	471
DUR3 (YHL016C)	20	105	53	116
HTA2 (YBL003C)	29	145	32	180
HTB2 (YBL002W)	122	559	184	617
ORF YNL300W	349	1281	340	1031
ORF YOL162W	44	160	66	151
ORF YLL057C	64	227	63	243
SVS1 (YPL163C)	129	453	112	370
ORF YOL163W	53	160	43	179
CAR2 (YLR438W)	49	138	43	165
TSL1 (YML100W)	148	399	175	373
PRY2 (YKR013W)	436	1148	472	1012
POL30 (YBR088C)	129	313	103	261
PDC6 (YGR087C)	129	276	78	270
ORF YOR247W	669	1372	485	1323

Floor=20, max-min>80,max/min>2, TEC1HC/*tec1*△>2 for both chip sets

CLAIMS

What is claimed is:

1. A method of inhibiting invasion of a host by a fungus, comprising contacting the fungus with a compound which inhibits expression of a gene expressed in the filamentation MAPK pathway and which enhances said pathway, in sufficient quantity that inhibition of the expression of said gene occurs, thereby inhibiting invasion of the host by the fungus.

2. A method according to Claim 1 wherein the gene is TOT10/YELO33W.

3. A method according to Claim 1 wherein the host is a plant and the compound is applied to a plant surface in such manner that it contacts the fungus.

4. A method according to Claim 1 wherein the fungus is a yeast.

5. A method of inhibiting invasion of a host by a fungus, comprising contacting the fungus with a compound which inhibits activity of a gene product encoded by a gene expressed in the filamentation MAPK pathway, in sufficient quantity that inhibition of the activity of said gene product occurs, thereby inhibiting invasion of the host by the fungus.

6. A method according to Claim 5 wherein the gene is TOT10/YELO33W.

7. A method according to Claim 5 wherein the host is a plant and the compound is applied to a plant surface in such manner that it contacts the fungus.

8. A method according to Claim 5 wherein the fungus is a yeast.

9. A method of identifying an agent which inhibits the filamentation MAPK pathway in a fungus, comprising the steps of:

   a) providing an expression vector comprising a nucleic acid molecule of a gene which is expressed in the filamentation MAPK pathway;

   b) transforming a suitable host cell with said expression vector under conditions suitable for expression of said gene;

   c) contacting said host cell with an agent to be tested; and

   d) comparing the expression of said gene in the presence of the agent with the expression of said gene in the absence of said agent,

   wherein if the expression of said gene is lower in the presence of the agent than in the absence of the agent, then the agent is an inhibitor of the filamentation MAPK pathway in a fungus.

10. A. method according to Claim 9, wherein the gene is TOT10/YELO33W.

11. A method according to Claim 9, wherein the fungus is yeast.

12. A method according to Claim 9, wherein the gene is identified by expression profiling as having repressed expression in the presence of galacturonic acid.

13. A method according to Claim 9, wherein the gene is identified by expression profiling as being expressed in haploid fungal cells and not expressed in diploid fungal cells.

14. A method of inhibiting fungal filamentation, comprising contacting the fungus with a compound which inhibits expression of a gene expressed in the filamentation MAPK pathway, in sufficient quantity that inhibition of the expression of said gene occurs, thereby inhibiting filamentation by the fungus.

15. A method of identifying an agent which modulates PGUI gene expression, comprising the steps of:

a) providing an expression vector comprising a nucleic acid molecule encoding PGUI;

b) transforming a suitable host cell with said expression vector under conditions suitable for expression of PGUI;

c) contacting said host cell with an agent to be tested; and

d) comparing the expression of PGUI in the presence of the agent with the expression of PGUI in the absence of said agent,

wherein a difference in the expression of PGUI in the presence of the agent as compared with in the absence of the agent indicates that the agent modulates PGUI expression.

16. A method of reducing the adverse effects of fungal invasion of a host, comprising administering to the host an effective amount of an agent which inhibits PGUI expression in the fungus.

17. A method of inhibiting invasion of a host by a fungus, comprising contacting the fungus with a compound which enhances expression of a gene expressed in the filamentation MAPK pathway and which inhibits said pathway, in sufficient quantity that enhancement of the expression of said gene occurs, thereby inhibiting invasion of the host by the fungus.

18. A method according to Claim 1 wherein the fungus is a yeast.

TARGETS OF THE MAP KINASE PATHWAY IN THE DEVELOPMENTAL SWITCH IN YEAST

ABSTRACT OF THE DISCLOSURE

Assessment of targets of the MAP kinase pathway in the developmental switch between haploid invasive growth and diploid pseudohyphal development in fungi, and identification of genes that show strong regulation by a MAPK pathway-specific transcription factor, Tec1, are described. Also described are methods of identifying an agent which inhibits the filamentation MAPK pathway in a fungus, and methods of inhibiting filamentation of a fungus or invasion of a host by a fungus.

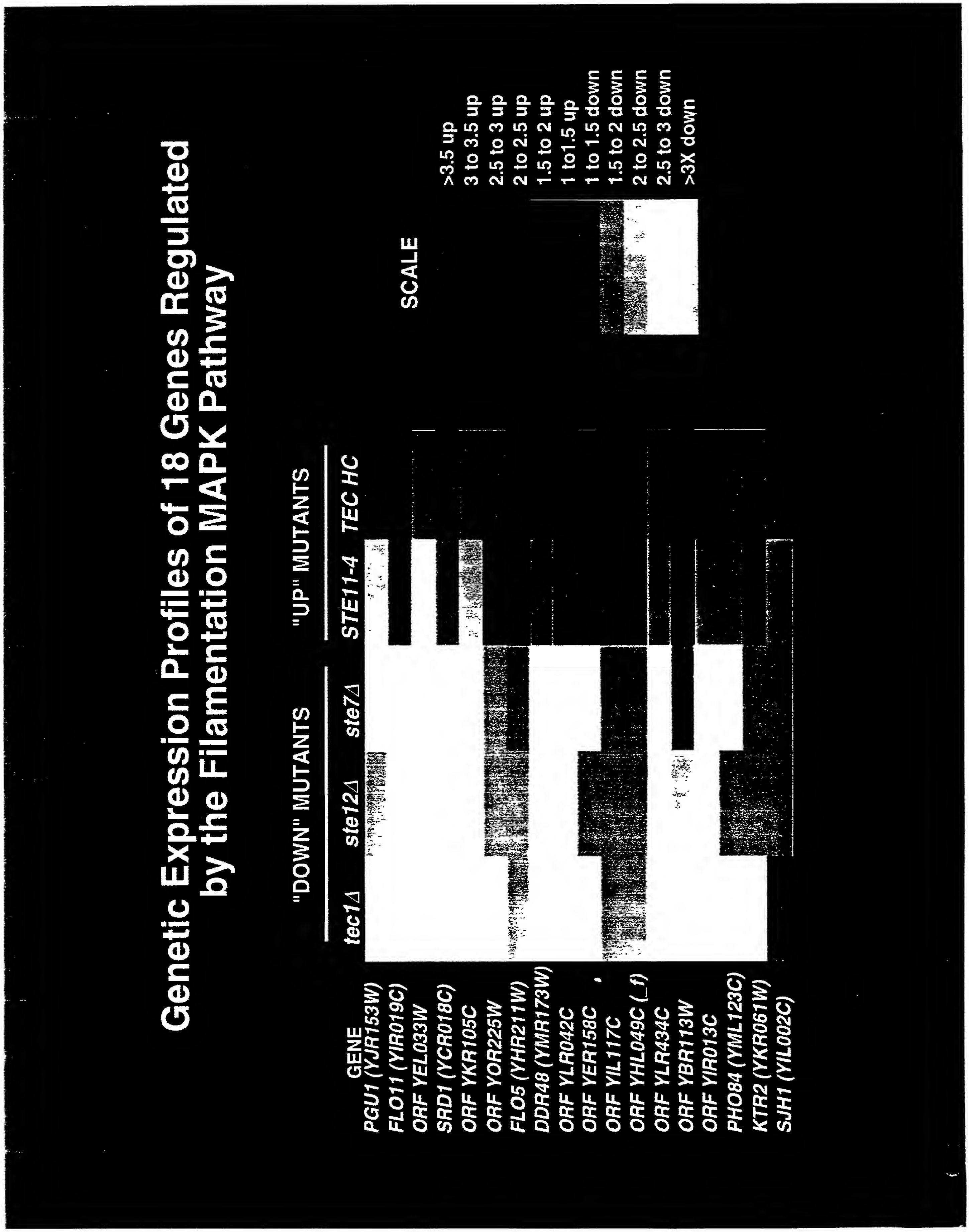
Genetic Expression Profiles of 18 Genes Regulated by the Filamentation MAPK Pathway
"DOWN" MUTANTS
"UP" MUTANTS
GENE
tec1Δ
ste12Δ
ste7Δ
STE11-4
TEC HC
PGU1 (YJR153W)
FLO11 (YIR019C)
ORF YEL033W
SRD1 (YCR018C)
ORF YKR105C
ORF YOR225W
FLO5 (YHR211W)
DDR48 (YMR173W)
ORF YLR042C
ORF YER158C
ORF YIL117C
ORF YHL049C (_f)
ORF YLR434C
ORF YBR113W
ORF YIR013C
PHO84 (YML123C)
KTR2 (YKR061W)
SJH1 (YIL002C)
SCALE
>3.5 up
3 to 3.5 up
2.5 to 3 up
2 to 2.5 up
1.5 to 2 up
1 to1.5 up
1 to 1.5 down
1.5 to 2 down
2 to 2.5 down
2.5 to 3 down
>3X down

Figure 1

MAPK Targets Include Proteins Known or Predicted to Enter the Secretory Pathway

PGU1	secreted endopolygalacturonase
FLO11	GPI-linked cell surface adhesion factor
TOT10/YEL033W	novel
SRD1	Zinc finger protein
TOT12/YKR105C	putative permease
TOT13/YOR225W	putative membrane protein
FLO5	GPI-linked cell surface adhesion factor
DDR48	cell surface protein
TOT11/YLR042C	GPI-linked cell surface protein
TOT7/YER158C	Homolog of mating morphogenesis protein Afr1
TOT8/YIL117C	Homolog of Chitin Synthase III subunit
TOT20/YHL049C	telomeric protein family member
TOT15/ YLR434C	novel
TOT14/YBR113W	putative membrane protein
TOT9/YIR013C	Zinc finger protein
PHO84	phosphate transporter, sugar permease family
KTR2	protein mannosyltransferase homolog
SJH1	Sac1-related inositol phosphate 5-phosphatase homolog

Figure 2

Sytematic Knockout Experiments

GENE	Haploid Invasion	Diploid Filamentation
PGU1	+++	+++
FLO11	-	-
TOT10/YEL033W	+	+
SRD1	ND	ND
TOT12/YKR105C	+++	+++
TOT13/YOR225W	+++	+++
FLO5	+++	+++
DDR48	+++	+++
TOT11/YLR042C	+++	+++
TOT7/YER158C	+++	+++
TOT8/YIL117C	+++	+++
TOT20/YHL049C	ND	ND
TOT15YLR434C	+++	+++
TOT14/YBR113W	+++	+++
TOT9/YIR013C	+++	+++
PHO84	+++	+++
KTR2	+++	+++
SJH1	+++	+++

Figure 3

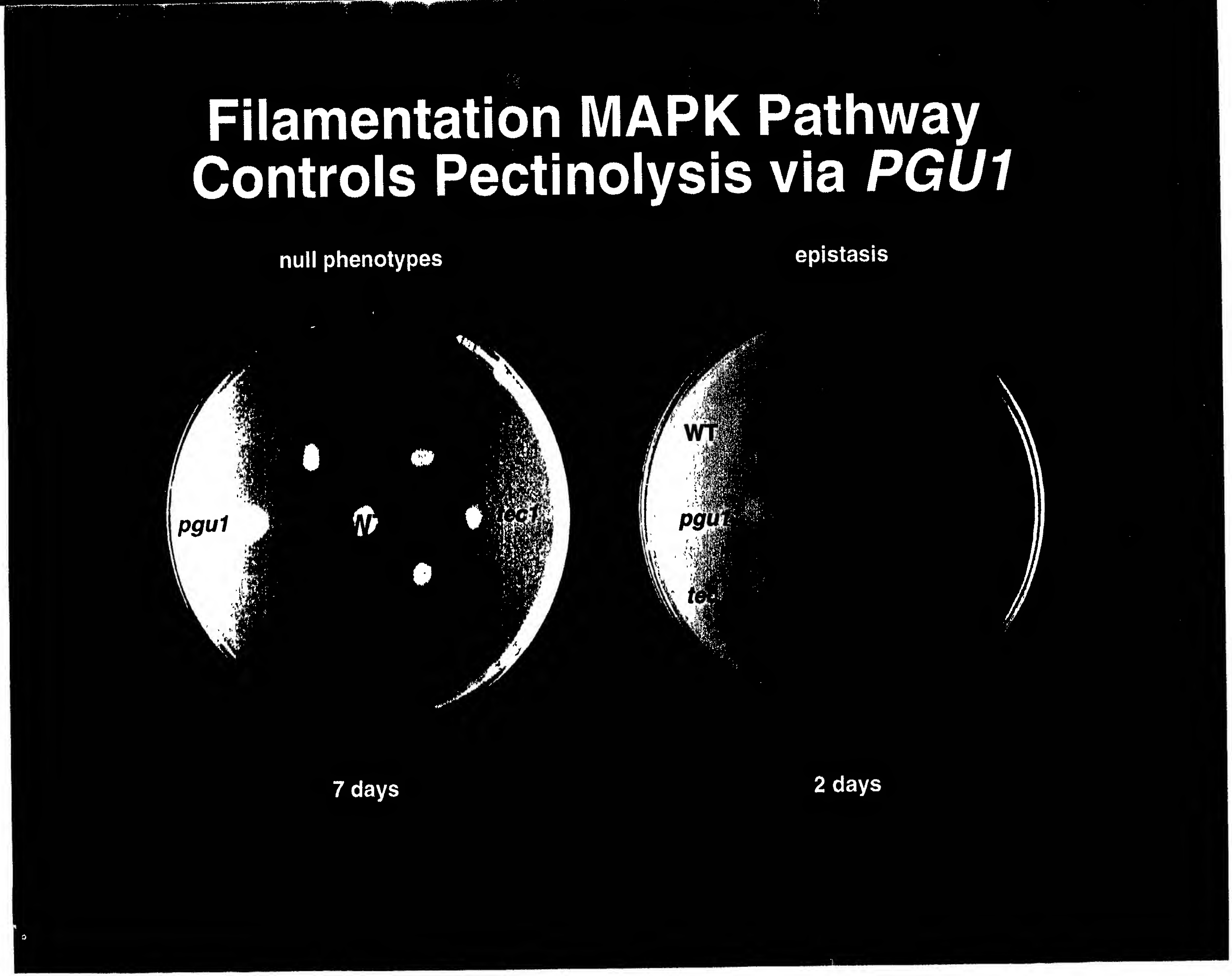
Filamentation MAPK Pathway Controls Pectinolysis via PGU1
null phenotypes
epistasis
pgu1
tec1
WT
7 days
2 days

Figure 4

Gene Induction by the Plant-Specific Carbohydrate Polygalacturonic Acid and Its Hydrolysis Product

Genes Selectively Induced by Polygalacturonic Acid

gene	GA/-	PGA/-	Protein Information
XBP1	2.40	6.65	Stress-induced transcriptional repressor
YHR217C (_f)	1.30	6.00	Protein of unknown function
YPL080C	2.80	5.70	Protein of unknown function
YPR098C	1.16	5.49	Protein of unknown function
YHL040C	2.04	5.00	Putative MFS Permease
YOL080C	1.35	4.74	Protein with similarity to Rnh70p and Pan2p
PHO84	1.39	4.70	phosphate transport, sugar permease homolog*
YMR293C	1.29	4.07	Protein with similarity to amidase
YLR184W	1.33	3.24	Protein of unknown function
YIL011W	1.01	3.01	Protein with similarity to PAU1 family
CYT1	1.02	2.82	Cytochrome c1
ATP11	1.29	2.65	F1-ATP synthase assembly protein
YOR091W	1.17	2.51	Protein of unknown function
PAU3	1.02	2.46	Stress-induced protein of the PAU1 family
SKO1	0.47	2.35	ATF/CREB transcriptional repressor
MSI4	0.73	2.06	Rab guanine nucleotide dissociation inhibitor

Regulated by Filamentation MAPK Pathway*

Genes Selectively Induced by Galacturonic Acid

gene	GA/-	PGA/-	Protein Information
VPS1	4.03	1.69	Vacuolar sorting protein, dynamin GTPase

Figure 5

Gene Repression by the Plant-Specific Carbohydrate Polygalacturonic Acid and its Hydrolysis Product

Genes Selectively Repressed by Polygalacturonic Acid

gene	GA/-	PGA/-	Protein Information
COP1/SEC33	0.63	0.17	alpha subunit of coatamer complex
YOL002C	1.36	0.18	Protein of unknown function
YDL173W	0.96	0.24	Protein of unknown function
COQ2	1.37	0.25	coenzyme Q (ubiquinone) biosynthesis
YIL176C (_f)	0.86	0.30	Protein with similarity to PAU1 family
YFL032W	0.80	0.30	Protein of unknown function
RPS33A	1.10	0.34	Ribosomal protein S28A
ARC35	1.25	0.39	Component of ARP2/3 complex
RPS26A	0.87	0.39	Ribosomal protein S26A
RPS10A	1.02	0.46	Ribosomal protein S10A

Genes Selectively Repressed by Galacturonic Acid

gene	GA/-	PGA/-	Protein Information
YEL033W	0.12	0.38	Protein of unknown function*
VID24	0.24	1.46	Vacuolar import and degradation of Fbp1
NDC1	0.29	1.02	Spindle pole body duplication factor
SKO1	0.47	2.35	ATF/CREB transcriptional repressor

*Regulated by the Filamentation MAPK Pathway

Figure 6

MAPK DATA	Note that the details of the list are slightly different from some of the figures because these data were floored and filtered slightly differently						
floor=20	data are sorted as TEC1-high copy/tec1Δ, Ty1 elements have been purged						
max-min>90							
scaling=bulk							
gene	WT	tec1	ste12	ste7	TEC1HC	STE11-4	YPD Title Line
STA1 (YIR019C)	262	47	45	173	742	2020	Cell surface flocculin
PGU1 (YJR153W)	50	20	34	21	269	28	Polygalacturonase, pectin hydrolyzing enzyme
ORF YEL033W	178	20	41	30	256	20	Protein of unknown function
DDR48 (YMR173W A)	492	147	147	148	1258	660	Stress protein induced by heat shock, DNA damage, or osmotic stress
ORF YLR042C	91	33	20	20	275	968	Protein of unknown function
SRD1 (YCR018C)	57	20	20	20	150	65	Nucleolar protein involved in pre rRNA processing, does not bind to small nucleolar RNA (snoRNA)
ORF YAR009C (_f)	657	90	165	336	657	1250	Protein of unknown function
ORF YHR214C-B exon 1 (_f)	261	59	60	75	403	544	Protein of unknown function
ORF YKR105C	112	20	44	45	118	74	Member of major facilitator superfamily (MFS) multidrug resistance (MFS MDF) protein family
ORF YOR225W	66	20	47	45	109	164	Protein of unknown function
ORF YLR434C	105	21	51	29	113	76	Protein of unknown function
ORF YBR113W	75	30	48	138	159	257	Protein of unknown function
ORF YAR009C (_i)	672	134	364	665	655	2273	Protein of unknown function
ORF YIR013C	69	26	20	20	120	51	Protein of unknown function
PHO84 (YML123C)	79	25	78	26	114	77	High affinity inorganic phosphate/H+ symporter, member of sugar permease family
ORF YAR009C (_r_i)	724	160	348	620	702	2168	Protein of unknown function
KTR2 (YKR061W)	68	30	68	59	128	146	Mannosyltransferase of KRE2 family, involved in N-linked glycosylation
ORF YMR173W	20	33	20	20	136	91	Stress protein induced by heat shock, DNA damage, or osmotic stress
TEC1 (YBR083W)	612	332	45	62	1201	223	Transcriptional activator involved with STE12 in pseudohyphal formation
DAN1 (YJR150C)	170	29	20	108	104	20	Protein induced during anaerobic growth
SOR1 (YJR159W) (_f)	132	65	119	163	233	208	Sorbitol dehydrogenase, converts L-sorbitol and NAD to L-sorbose and NADH (SOR1 and YDL246C code for nearly identical proteins)
ORF YIL117C	20	20	20	20	69	195	Protein of unknown function
ORF YHR214C-B exon 2 (_f)	305	137	167	162	472	267	Protein of unknown function
ORF YNL256W	59	34	87	55	117	60	Protein with similarity to bacterial dihydropteroate synthase
GAP1 (YKR039W)	401	126	327	304	424	406	General amino acid permease, proton symporter transporter for all naturally occurring L amino acids, 4-aminobutyric acid (GABA), ornithine, citrulline, some D amino acids, and some L-[illegible]
ORF YFL030W	20	26	38	47	87	175	Protein with similarity to Methanobacterium aspartate transaminase
ORF YLR152C	57	33	75	73	110	159	Protein with similarity to E. m3p
ORF YGL088W	97	35	20	27	113	20	Protein with similarity to Cyanophora paradoxa ribosomal protein se[illegible]
GPM2 (YDL021W)	68	20	74	69	63	131	Phosphoglycerate mutase, with similarity to Gpm1p and Gpm3p
ORF YFR024C A exon 1	48	20	105	101	83	20	Protein with similarity to Ysc84p, Rvs167p, Abp1p, and Sla1p
PPG1 (YNR032W)	44	41	42	54	127	89	Protein serine/threonine phosphatase involved in glycogen accumulation, member of the PPP family of protein phosphatases and related to PP2A phosphatases
GCY1 (YOR120W)	45	27	89	54	78	180	Galactose induced protein with strong similarity to crystallin protein of vertebrate eye lens
RHK1 (YBL082C)	153	57	133	121	164	136	Mannosyltransferase involved in N glycosylation, Hansenula mrakii HM 1 killer toxin resistance protein
ORF YHL049C (_f)	20	20	20	20	57	107	Protein with similarity to other subtelomerically encoded proteins including Yer189p, Yml133p, and Yil177c, encoded from a subtelomeric Y' region
MUP3 (YHL036W)	54	31	146	70	86	56	Low affinity methionine amino acid permease
MUP1 (YGR055W)	179	94	315	280	257	192	High affinity methionine permease
ORF YJR025C	1042	655	697	731	1731	1531	3-hydroxyanthranilate 3,4 dioxygenase, involved in biosynthesis of nicotinic acid from tryptophan
ORF YDR111C	153	44	54	40	116	23	Protein with similarity to alanine aminotransferase (CP 248758)
TCI1 (YDR161W)	104	39	137	133	96	87	Protein that interacts with protein phosphatase 2C
PDE2 (YOR360C)	102	61	118	96	149	110	3',5'-cyclic nucleotide phosphodiesterase
CWP1 (YKL096W)	149	76	176	166	181	430	Mannoprotein of the cell wall, member of the PAU1 family
ORF YOL164W	67	39	90	121	92	81	Protein with similarity to Pseudomonas sp. alkyl sulfatase
ORF YAR066W (_f)	98	86	36	49	202	101	Protein of unknown function (YAR066W and YHR214W code for identical proteins)
AIP2 (YDL178W)	209	87	217	135	197	128	Actin interacting protein and homolog of D lactate dehydrogenase Dld1p
ORF YGR257C	87	42	176	120	95	103	Protein with similarity to members of the mitochondrial carrier family (MCF)
RFC3 (YNL290W)	47	47	20	20	105	20	Replication factor C, 40 kDa subunit, member of the DEAD box family
ORF YER049W	255	106	176	125	236	105	Protein of unknown function
TIP1 (YBR067C)	2232	1574	159	182	3483	2891	Cold and heat shock induced mannoprotein of the cell wall, member of the PAU1 family
ORF YLR231C	178	76	144	152	168	445	Protein with weak similarity to rat kynureninase (PIR PS0370), likely active in tryptophan degradation and nicotinic acid synthesis
PCL1 (YNL289W)	53	39	59	59	84	185	G1/S specific cyclin that can interact with the Cdc28p-like kinase Pho85p
ARG1 (YOL058W)	779	460	1418	1405	971	1999	Argininosuccinate synthetase (citrulline: aspartate ligase)
ORF YOR248W (_f)	887	842	717	632	1775	943	Protein of unknown function
FTR1 (YER145C)	239	200	429	230	415	383	Iron permease that mediates high affinity iron uptake
ORF YNR018W	1947	1008	2056	1789	2089	2619	Protein of unknown function
CIT2 (YCR005C)	1555	820	1815	1324	1697	1682	Citrate synthase, peroxisomal, converts citrate plus CoA into acetyl CoA and oxaloacetate
ORF YOR292C	49	66	59	47	135	86	Protein of unknown function
ORF YEL006W	105	63	119	85	127	43	Protein with similarity to Aac1p, Pet9p, Aac3p, Yil006p and other members of the mitochondrial carrier (MCF) family
SVS1 (YPL163C)	650	426	337	274	858	1692	Serine and threonine rich protein required for vanadate resistance
ELO1 (YJL196C)	765	607	670	695	1218	1022	Fatty acid elongation protein involved in elongation of tetradecanoic acid to hexadecanoic acid
ORF YJL145W	165	174	253	148	349	337	Protein of unknown function
ORF YHR108W	60	20	114	91	40	112	Protein of unknown function
VID24 (YBR105C)	127	63	85	115	126	400	Protein required for vacuolar import and degradation of Fbp1p (Hui-Lin Chiang, pers. comm.)
ORF YOR389W	67	38	77	122	76	80	Protein of unknown function
LSC2 (YGR244C)	75	45	146	109	90	260	Beta subunit of succinyl CoA synthetase (succinyl CoA ligase) (succinate thiokinase)
CLP1 (YOR250C)	63	41	124	131	82	142	Subunit of cleavage and polyadenylation factor IA, required for 3' end processing of pre mRNA

Figure 7

NAME=GA					
gene	YPD	GA	PGA	GA/YPD	PGA/YPD
ORF YIL101C	20	48	133	2.40	6.65
ORF YLR344W exon 1 (_i)	33	138	208	4.18	6.30
ORF YHR217C (_r_i)	20	29	120	1.45	6.00
ORF YHR217C (_f)	20	26	120	1.30	6.00
ORF YPL080C	20	56	114	2.80	5.70
ORF YPR098C	37	43	203	1.16	5.49
ORF YHL040C	27	55	135	2.04	5.00
ORF YOL080C	23	31	109	1.35	4.74
PHO84 (YML123C)	33	46	155	1.39	4.70
ORF YMR293C	28	36	114	1.29	4.07
ORF YLR184W	66	88	214	1.33	3.24
ORF YIL011W	153	154	460	1.01	3.01
ORF YJR027W exon 2 (_f)	156	351	459	2.25	2.94
CYT1 (YOR065W)	91	93	257	1.02	2.82
ORF YLL025W (_f)	251	355	693	1.41	2.76
ORF YML039W exon 2 (_f)	192	486	524	2.53	2.73
ATP11 (YNL315C)	51	66	135	1.29	2.65
ORF YOR091W	59	69	148	1.17	2.51
ORF YMR143W exon 1 (_i)	385	895	964	2.32	2.50
ORF YJR029W exon 2 (_f)	71	95	175	1.34	2.46
PAU3 (YCR104W) (_f)	180	184	443	1.02	2.46
SKO1 (YNL167C)	43	20	101	0.47	2.35
PRE3 (YJL001W) exon 1	112	166	241	1.48	2.15
ORF YMR045C exon 2 (_f)	93	132	197	1.42	2.12
ORF YNL006W	77	100	161	1.30	2.09
MSI4 (YOR370C)	63	46	130	0.73	2.06
ORF YPR139C	91	122	187	1.34	2.05
SPO15 (YKR001C)	35	141	59	4.03	1.69
HHO1 (YPL127C)	81	45	135	0.56	1.67
ILV3 (YJR016C)	119	72	182	0.61	1.53
ORF YBR105C	82	20	120	0.24	1.46
LYS4 (YDR234W)	479	244	576	0.51	1.20
ORF YOR009W	144	310	158	2.15	1.10
NDC1 (YML031W)	113	33	115	0.29	1.02
ORF YOL073C	143	180	88	1.26	0.62
ORF YJL223C (_f)	134	175	81	1.31	0.60
ORF YMR242C	1170	1227	610	1.05	0.52
ORF YOR248W (_f)	497	422	239	0.85	0.48
ORF YPL081W exon 1	159	135	75	0.85	0.47
ORF YML019W	177	91	82	0.51	0.46
ORF YOR293W exon 1 (_f)	3170	3237	1446	1.02	0.46
ORF YMR050C exon 1 (_f)	374	263	169	0.70	0.45
RPS26A (YGL189C)	11511	9978	4526	0.87	0.39
ORF YNR035C	200	249	78	1.25	0.39
ORF YEL033W	172	20	66	0.12	0.38
TSL1 (YML100W)	103	20	39	0.19	0.38
RPS33A (YOR167C) (_f)	1726	1899	589	1.10	0.34
ORF YFL032W	122	98	37	0.80	0.30
ORF YIL176C (_f)	115	99	34	0.86	0.30
COQ2 (YNR041C)	84	115	21	1.37	0.25
ORF YDL173W	141	136	34	0.96	0.24
ORF YOL002C	111	151	20	1.36	0.18
ORF YDL145C	118	74	20	0.63	0.17
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Figure 8

NAME=GA					
gene	YPD	GA	PGA	GA/YPD	PGA/YPD
ORF YLR344W exon 1 (_i)	33	138	208	4.18	6.30
SPO15 (YKR001C)	35	141	59	4.03	1.69
ORF YPL080C	20	56	114	2.80	5.70
ORF YML039W exon 2 (_f)	192	486	524	2.53	2.73
ORF YIL101C	20	48	133	2.40	6.65
ORF YMR143W exon 1 (_i)	385	895	964	2.32	2.50
ORF YJR027W exon 2 (_f)	156	351	459	2.25	2.94
ORF YOR009W	144	310	158	2.15	1.10
ORF YHL040C	27	55	135	2.04	5.00
PRE3 (YJL001W) exon 1	112	166	241	1.48	2.15
ORF YHR217C (_r_i)	20	29	120	1.45	6.00
ORF YMR045C exon 2 (_f)	93	132	197	1.42	2.12
ORF YLL025W (_f)	251	355	693	1.41	2.76
PHO84 (YML123C)	33	46	155	1.39	4.70
COQ2 (YNR041C)	84	115	21	1.37	0.25
ORF YOL002C	111	151	20	1.36	0.18
ORF YOL080C	23	31	109	1.35	4.74
ORF YPR139C	91	122	187	1.34	2.05
ORF YJR029W exon 2 (_f)	71	95	175	1.34	2.46
ORF YLR184W	66	88	214	1.33	3.24
ORF YJL223C (_f)	134	175	81	1.31	0.60
ORF YHR217C (_f)	20	26	120	1.30	6.00
ORF YNL006W	77	100	161	1.30	2.09
ATP11 (YNL315C)	51	66	135	1.29	2.65
ORF YMR293C	28	36	114	1.29	4.07
ORF YOL073C	143	180	88	1.26	0.62
ORF YNR035C	200	249	78	1.25	0.39
ORF YOR091W	59	69	148	1.17	2.51
ORF YPR098C	37	43	203	1.16	5.49
RPS33A (YOR167C) (_f)	1726	1899	589	1.10	0.34
ORF YMR242C	1170	1227	610	1.05	0.52
PAU3 (YCR104W) (_f)	180	184	443	1.02	2.46
CYT1 (YOR065W)	91	93	257	1.02	2.82
ORF YOR293W exon 1 (_f)	3170	3237	1446	1.02	0.46
ORF YIL011W	153	154	460	1.01	3.01
ORF YDL173W	141	136	34	0.96	0.24
RPS26A (YGL189C)	11511	9978	4526	0.87	0.39
ORF YIL176C (_f)	115	99	34	0.86	0.30
ORF YOR248W (_f)	497	422	239	0.85	0.48
ORF YPL081W exon 1	159	135	75	0.85	0.47
ORF YFL032W	122	98	37	0.80	0.30
MSI4 (YOR370C)	63	46	130	0.73	2.06
ORF YMR050C exon 1 (_f)	374	263	169	0.70	0.45
ORF YDL145C	118	74	20	0.63	0.17
ILV3 (YJR016C)	119	72	182	0.61	1.53
HHO1 (YPL127C)	81	45	135	0.56	1.67
ORF YML019W	177	91	82	0.51	0.46
LYS4 (YDR234W)	479	244	576	0.51	1.20
SKO1 (YNL167C)	43	20	101	0.47	2.35
NDC1 (YML031W)	113	33	115	0.29	1.02
ORF YBR105C	82	20	120	0.24	1.46
TSL1 (YML100W)	103	20	39	0.19	0.38
ORF YEL033W	172	20	66	0.12	0.38
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Figure 9

Homologous Genes Induced by Filamentation and Mating MAPK Pathways

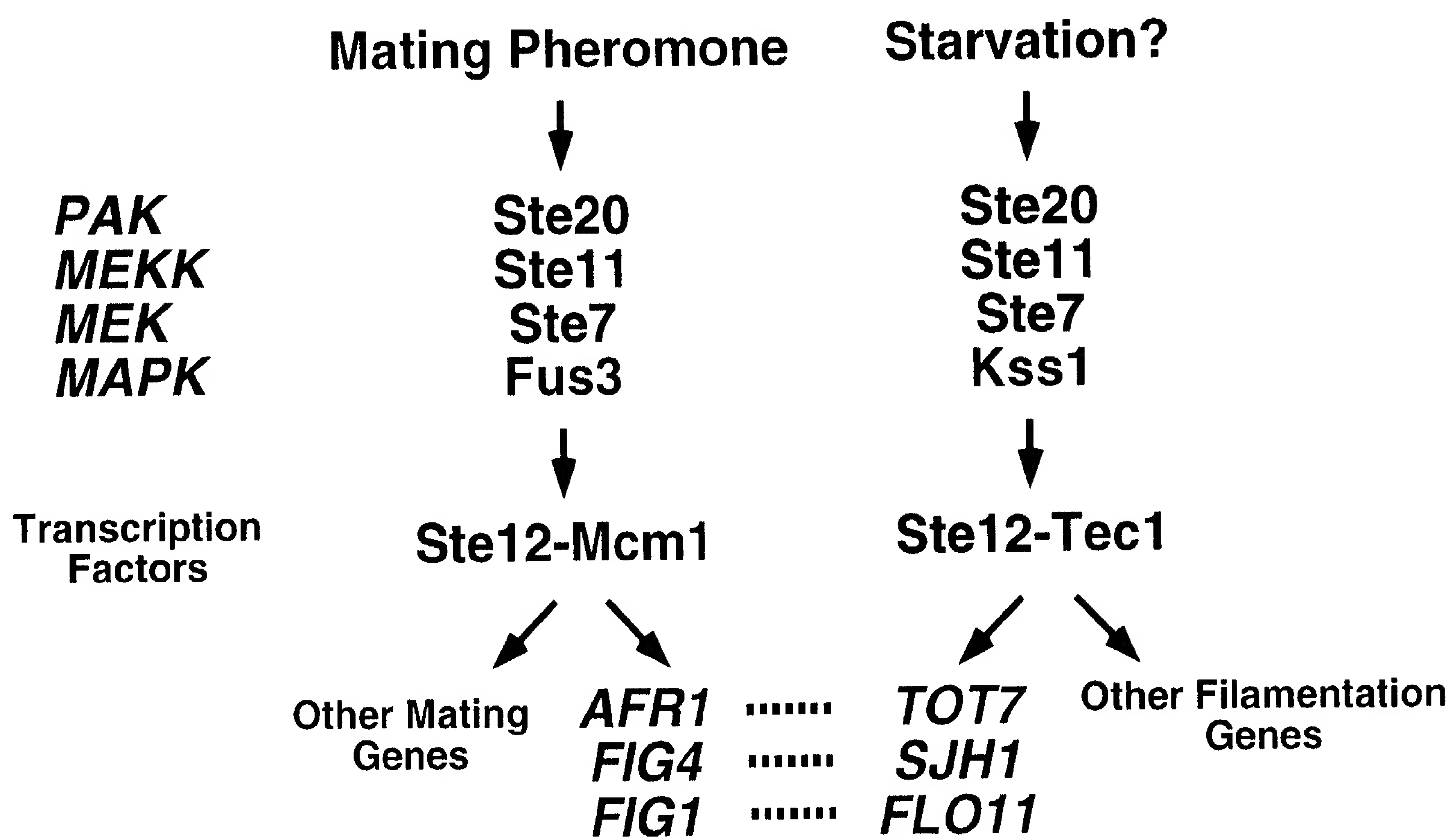

Figure 10

gene	WT	tec1	ste12	ste7	TEC1HC	STE11-4	YPD Title Lines
MFA1 (YDR461W)	3803	5138	20	28	3378	2262	Mating pheromone a-factor, exported from cell by Ste6p
ANB1 (YJR047C)	1285	1668	31	110	1090	39	Translation initiation factor eIF5A, contains essential hypusine modification
HIS3 (YOR202W)	334	317	34	60	378	73	
MFA2 (YNL145W)	7879	6017	821	1993	4780	4674	Mating pheromone a-factor, exported from cell by Ste6p
STE2 (YFL026W)	866	1169	121	178	802	598	Pheromone alpha-factor receptor, seven-transmembrane domain protein
ORF YDL120W	118	102	20	49	117	59	Mitochondrial protein involved in respiratory function and iron homeostasis, homolog of human frataxin which is defective in Friedreich's ataxia
ORF YIL121W	286	169	52	119	212	258	Member of major facilitator superfamily (MFS) multidrug-resistance (MFS-MDR) protein family
ORF YOL026C	108	140	20	28	127	83	Protein of unknown function
ORF YKL120W	906	714	168	217	741	1989	Protein with similarity to members of the mitochondrial carrier (MCF) family
COS6 (YGR295C)	639	480	122	131	415	130	Protein with strong similarity to other subtelomerically-encoded proteins such as Cos5p, Ybr302p, Cos3p, Cos1p, Cos4p, Cos8p, Cos6p, Cos9p
CHS2 (YBR038W)	104	76	20	20	60	60	Chitin synthase II, responsible for primary septum disk
ORF YLR437C	103	48	20	20	58	34	Protein of unknown function
QRI2 (YDL105W)	102	48	21	20	79	20	Protein of unknown function
GPA1 (YHR005C)	168	124	37	51	75	39	Guanine nucleotide-binding protein alpha subunit of the pheromone response pathway
ORF YKL137W	117	59	27	30	67	55	Protein of unknown function
AGA2 (YGL032C)	299	538	69	64	342	1990	a-Agglutinin binding subunit
ORF YHR214W (_f)	107	64	25	25	104	114	Protein of unknown function (YAR066W and YHR214W code for identical proteins)
LEU1 (YGL009C)	2205	1865	519	489	1758	3671	3-Isopropylmalate dehydratase, second step in leucine biosynthesis pathway
PDR12 (YPL058C)	198	172	47	62	115	134	Protein with similarity to Pdr5p and Snq2p, member of the ATP-binding cassette (ABC) superfamily
FAR1 (YJL157C)	258	244	65	52	151	357	Inhibitor of Cdc28p-Cln1p and Cdc28p-Cln2p kinase complexes involved in cell cycle arrest for mating
MEP2 (YNL142W)	156	115	40	33	227	123	Ammonia permease of low capacity and high affinity
ILV3 (YJR016C)	979	785	256	268	1012	1158	Dihydroxyacid dehydratase (DAD), third step in valine and isoleucine biosynthesis pathway
NOP4 (YPL043W)	114	98	30	29	107	28	Nucleolar protein required for ribosome biogenesis, has 3 canonical RNA recognition (RRM) domains and one degenerate RNA recognition (RRM) domain
ADE5,7* (YGL234W)	511	313	137	114	467	107	Phosphoribosylamine-glycine ligase (GARSase) + Phosphoribosylformylglycinamidine cyclo-ligase (AIRSase), bifunctional protein
ORF YMR304C-A	72	86	20	20	107	38	
MSB2 (YGR014W)	71	59	20	20	110	27	Protein for which overproduction suppresses bud emergence defect of cdc24 mutant
MPT5 (YGL178W) exon 1	129	89	37	20	71	45	Protein required for high temperature growth, recovery from alpha-factor arrest, and normal lifespan of yeast cells
WCS2 (YNL283C)	251	190	72	125	257	200	Protein required for maintenence of cell wall integrity and for the stress response
RPS24B (YLR367W) exon 1	617	747	186	277	651	632	Ribosomal protein S22B (yeast S24) (rp50) (YS22)(rat S15A) (RPS22A and RPS22B code for nearly identical proteins)
STE6 (YKL209C)	143	143	44	41	134	302	Membrane transporter of ATP-binding cassette (ABC) superfamily responsible for export of "a" factor mating pheromone
BAR1 (YIL015W)	148	165	46	20	120	529	Secreted pepsin-like protease that degrades alpha-factor (barrpepsin)
CHA1 (YCL064C)	77	124	24	21	78	20	L-serine/L-threonine deaminase (catabolic L-serine/L-threonine dehydratase)
ORF YER150W	678	374	217	221	452	623	Protein with similarity to Sed1p
ORF YMR305C	1667	1494	541	601	1423	1049	Protein with similarity to Bgl2p and other glucans (GB Z49212)
ORF YPR156C	340	152	112	114	115	233	Member of major facilitator superfamily (MFS) multidrug-resistance (MFS-MDR) protein family
FET4 (YMR319C)	245	310	81	78	324	159	Low-affinity Fe(II) transport protein
MEP3 (YPR138C)	168	107	56	57	153	129	Ammonia permease of high capacity and low affinity
ORF YBR214W	117	94	39	43	126	141	Protein with similarity to moc1 protein of S. pombe
ORF YGR151C (_i)	398	421	134	130	487	146	Protein of unknown function
FUS3 (YBL016W)	248	176	84	65	318	513	Serine/threonine protein kinase of the MAP kinase family required for cell cycle arrest and for cell fusion during mating
ORF YOR203W	347	349	119	131	384	292	Protein of unknown function

Figure 11